

## Conditioning and aversion to toxic *Solanum bonariense* (“naranjillo”) leaves in calves

### Condicionado e aversão a folhas tóxicas de *Solanum bonariense* (“naranjillo”) em bezerros

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#### ABSTRACT

*Solanum bonariense* is a perennial poisonous shrub that induces cerebellar cortical degeneration when eaten by cattle. The aim of this research was to outline a protocol to induce a conditioned aversion to this plant. During the pre-conditioning period ten calves (126±12kg BW) were maintained at half of their normal energy intake with lucerne hay and water ad libitum, to stimulate consumption of *S. bonariense*. Every two days they were offered 100g of *S. bonariense* leaves for 5 minutes. Calves began eating the target plant on day 10 and consumed all the plant material on day 12. The conditioning period began after each calf consumed the entire amount of *S. bonariense* for three consecutive sessions. Five animals were randomly selected for conditioning, and after ingestion of *S. bonariense* they were dosed by oral gavage with lithium chloride (LiCl) at 200mg kg<sup>-1</sup> BW (treated group), while the other five received a similar volume of water by oral gavage (control group). After 2 doses of LiCl the treated group ate no *S. bonariense* while the control group consumed the entire 100g. We confirmed that LiCl is a powerful tool to induce conditioned aversions against *S. bonariense* in calves, which persists for at least 3 months.

**Key words:** *Solanum bonariense*, poisonous plants, conditioned taste aversion, calves.

#### RESUMO

*Solanum bonariense* é uma planta tóxica, arbustiva e perene que induz a degeneração cortical cerebelar quando ingerida por bovinos. O presente trabalho objetiva criar um protocolo para induzir aversão condicionada para essa planta em bovinos. Durante o período de adaptação às condições experimentais, dez bezerros (126±12kg) foram mantidos com metade de seu fornecimento alimentar energético normal que incluiu feno de alfafa e água ad libitum, para estimular o consumo de *S. bonariense*. A cada dois dias, foram fornecidas oralmente

100g de folhas de *S. bonariense* por cinco minutos. Os bezerros começaram a ingerir a planta no 10º dia de experimento e consumiram toda a planta no 12º dia. O período condicionado começou após cada bezerro ter consumido a quantidade total de *S. bonariense* por três sessões consecutivas. Cinco animais foram aleatoriamente selecionados para aversão condicionada e, após ingestão da planta, receberam solução oral de 200mg kg<sup>-1</sup> de peso vivo de cloreto de lítio (LiCl) (grupo tratado), enquanto que os outros cinco bovinos receberam dosagem oral com volume similar de solução fisiológica (grupo controle). Após duas doses de LiCl, o grupo tratado não comeu folhas de *S. bonariense*, enquanto que o grupo controle consumiu toda a quantidade fornecida. Os resultados do presente trabalho demonstram que LiCl é uma ferramenta útil para induzir aversão condicionada contra *S. bonariense* em bezerros, que persiste por pelo menos 3 meses.

**Palavra-chave:** *Solanum bonariense*, plantas tóxicas, aversão condicionada, bezerros.

#### INTRODUCTION

Toxic plants are a serious problem in Uruguay (RIET-CORREA & MEDEIROS, 2001) and worldwide. In Uruguay, an annual death of 98.000 livestock heads was estimated that is due to toxic plants (RIET-CORREA & MEDEIROS, 2001). There are at least 31 toxic plant species in Uruguay which belong to 26 genera. *Solanum bonariense* is a perennial native shrub that is widespread in pastures in Uruguay, Northern Argentina, and Southern Brazil. Although the plant is not highly palatable, it is often consumed by grazing cattle when more desirable forage

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is reduced due to drought or overgrazing (VERDES et al., 2006). When consumed by cattle for extended periods of time, ingestion results in cerebellar cortical degeneration (RIET-CORREA et al., 1983; VERDES et al., 2006). The toxin(s) in the plant is not known, but appears to cause a glycolipid-type storage disease in Purkinje cells (VERDES et al., 2015). This debilitating neurological condition has a low mortality rate but high morbidity in affected animals, as the lesions are permanent (VERDES et al., 2006).

Grazing herbivores normally assess the nutritional value of available pasture and range forages, and generally select a more nutritious diet that is available within a particular environment. Besides selecting nutritious diets, grazing animals generally avoid plants that cause toxicosis or malnutrition (PROVENZA et al., 1992; DUNCAN & YOUNG, 2002). This selective ability is based on the relationship between the forage flavor (taste and smell) and their post-ingestion nutritional consequences (DUNCAN & YOUNG, 2002). Animals at times over-ingest toxic plants for reasons that are very complex (PROVENZA et al., 1992). Even so, it is possible to modify herbivore diet selection and improve their diet through the association of a harmful substance with gastric discomfort (PROVENZA, 1996). This process of conditioning as taste aversion can be a useful behavioral tool to train livestock to avoid some toxic plants (RALPHS & PROVENZA, 1999). Typically aversions are conditioned in livestock using lithium chloride (LiCl) (BURRITT et al., 1990; PROVENZA, 1996; RIET-CORREA & MEDEIROS, 2001; PFISTER et al., 2002; ALMEIDA et al., 2009). The LiCl is currently the most widely-used emetic in behavioral studies with animals and in human clinical applications (RALPHS & PROVENZA, 1999). It causes nausea without dangerous side-effects (PROVENZA et al., 1994; RALPHS & PROVENZA, 1999). The different methods of administering LiCl (mixed in food, orally, bolus or subcutaneous or intraperitoneal injections) appear equally effective in creating an aversion (NACHMAN & ASH, 1973; RALPHS & PROVENZA, 1999). As a result of its caustic nature the relatively large quantities required to create aversions in livestock (80–200mg/kg BW) must be administered into the rumen either orally in solution or in boluses, allowing dilution in rumen fluid (RALPHS & PROVENZA, 1999). Li is retained at significant levels in the body for up to 96 h (RALPHS, 1999). Treated cattle are most severely ill, at second day

after dosing, requiring a recovery period of at least 3 d (RALPHS & PROVENZA, 1999). Aversive conditioning with LiCl works similarly in goats, horses, sheep and cattle (RALPHS & OLSEN, 1990; 1992; RALPHS & CHENEY, 1993; RALPHS et al., 1994; PFISTER & PRICE, 1996; PFISTER et al., 2002; DUNCAN & YOUNG, 2002), and has been used to train animals to avoid toxic plants that may vary greatly in palatability (RALPHS et al., 1994; DUMONT & BOISSY, 1999; MANUELIAN et al., 2010; 2014).

The aim of this research was to establish whether LiCl could be used to induce a conditioned aversion in calves to recently harvested leaves of *Solanum bonariense*.

## MATERIALS AND METHODS

### Location and animals

The experiment was approved by the Facultad de Veterinaria (UdelaR) IACUC, and performed at the Facultad de Veterinaria (UdelaR) Experimental Farm No. 2 (Libertad, San José, Uruguay, 34°40'S, 56°35'W). Ten crossbred (Holstein x Jersey) males calves (126±12kg BW) were used; all animals were maintained from birth until their inclusion in the experimental protocol in a *S. bonariense*'s free dairy farm, to ensure the same initially naïve condition to the target plant in the group. Five animals were randomly selected to receive the aversion treatment (LiCl, 200mg kg<sup>-1</sup> orally) while the other five received the same volume of water (control group).

### Plant and lithium chloride (LiCl)

*Solanum bonariense* was collected at the Veterinary School (UdelaR) at Montevideo, Uruguay (34°54'S, 56°08'W), according to VERDES et al. 2006. Recently harvested fresh leaves from several different plants were offered to animals in all the trials. LiCl (Sigma® L4408, St. Louis, USA) was administered in an oral gavage with a final dose of 200mg kg<sup>-1</sup> BW, according to BURRITT et al. (1990). The LiCl doses at this concentration (BURRITT & PROVENZA, 1990), and 100g of leaves of *S. bonariense* (VERDES et al., 2006) did not adversely affect the health of treated animals.

### Experimental Protocol

Diet to stimulate the consumption of plant (preconditioning): As in other cases of poisonous plants, *S. bonariense* is consumed by cattle mainly during periods of forage scarcity. Since *S. bonariense*

is naturally unpalatable, we emulated natural forage scarcity by holding animals in a paddock with water and reduced access to hay. Calves were maintained on a diet of lucerne hay, which was equivalent to 50% of Metabolic Energy (ME) for maintenance requirements for this age and size (Table 1), (ROHWEDER et al., 1978). All animals were observed daily by caretakers, and weighed weekly to avoid significant weight losses which can affect animal welfare.

#### Conditioning protocol

To familiarize the animals with the plant, 100g of recently harvested leaves were offered individually to each animal every two days at 0800h for a 5min period after an overnight fast (consumption test). Refusals were weighed. Conditioning began when all calves ate the entire of 100g of *S. bonariense* leaves for three consecutive consumption tests. After that conditioning, five calves were randomly chosen and given an oral dose of LiCl (200mg kg<sup>-1</sup> BW) by gavage immediately after eating the leaves. They were then isolated without food for 30min, and later returned to the group with access to food; this procedure was repeated twice (i.e., maximum of 2 doses of LiCl). The five calves that constituted the control group were dosed with the same volume of water (on a weight basis) simultaneously with the treated group. Three months after the conclusion of the conditioning trials, a final assessment was made to determine if the LiCl-treated calves were still averted. They were again offered 100g of recently harvested leaves to each animal for a 5min period after an overnight fast. Refusals were weighed.

#### Statistical analysis

A mixed model repeated measures analysis was done using SAS 9.3 to compare consumption of *S. bonariense* over time after dosing with LiCl.

Table 1 - Chemical analysis of lucerne hay fed to calves to simulate a scarce forage situation and calculated to provide 50% of their Metabolic Energy (ME) maintenance requirements.

COMPONENT	% fresh basis	% DM basis
Dry matter	90.27	---
Ash	6.28	6.96
Neutral detergent fiber	57.70	63.92
Acid detergent fiber	42.53	47.11
Crude Protein	10.74	11.90

ME (Mcal kg<sup>-1</sup> DM)= 1.881 (ROHWEDER et al., 1978).

The model included individual animals nested within treatment, test days, and the day x treatment interaction. Animals were a random factor in the model, and the covariance structure of the mixed model was selected based on the best fitting model. Means for the test day x treatment interaction were compared using the Pdiff option in SAS 9.3.

## RESULTS

#### Plant consumption

Animals were provided with a lucerne-based diet (Table 1) equivalent to 50% of their metabolic energy requirements calculated from NRC (2000) tables. After 10 days, some calves began to consume *S. bonariense* leaves in small amounts, and on day 12, all animals consumed the total amount of offered leaves (Figure 1).

Conditioned taste aversion: There was a day x treatment interaction (P=0.01) for consumption of *S. bonariense* leaves (Figure 1). Dosing with LiCl decreased (P=0.004) the consumption of *S. bonariense* leaves by about 40% in the treated calves after one pairing when tested 48 hours later, compared to control animals which ate all of the offered plant material. Treated and control animals differed on all subsequent test days (P<0.001), as LiCl-treated animals completely refused to eat *S. bonariense* leaves after a second conditioning trial, whereas the control animals ate all of the proffered material (Figure 2). Three months after finishing the trial, the treated animals maintained the aversion and refused to eat *S. bonariense* leaves.

## DISCUSSION

*Solanum bonariense* is present in pastures, and remains toxic, throughout the year in Uruguay regardless of season (VERDES et al., 2006), thus the plant is a threat to intoxicate cattle whenever conditions favor consumption by livestock. This study

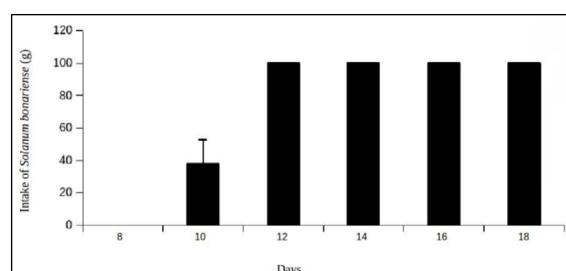


Figure 1 - Ingestion (g ± SEM) of *S. bonariense* during the conditioning period in calves.

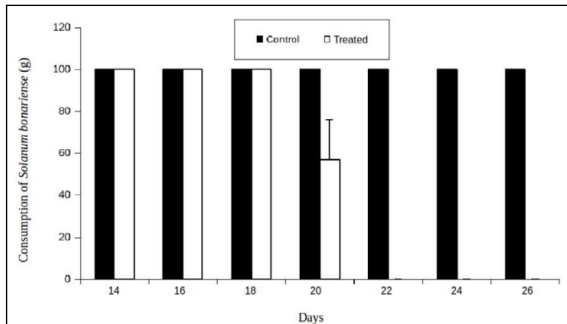


Figure 2 - Intake (g ± SEM) of *S. bonariense* in trials to train animals to consume the plant (days 14-18) and to evaluate a conditioned aversion (days 20-26). From days 22 to 26 there was no consumption in the treated group, and a complete refusal of *S. bonariense* leaves was confirmed in treated calves 3 months after finishing this trial (not shown).

demonstrated that LiCl at 200mg kg<sup>-1</sup> was an effective and safe tool for inducing a conditioned aversion against *S. bonariense* in calves. Our results confirmed previous reports on the effect of LiCl as an inducer of aversion to palatable foods in ruminants (BURRITT & PROVENZA, 1990; RUIZ & VERDES, 2010). Conditioned aversion has been successfully used to condition cattle (RALPHS & OLSEN, 1992), sheep (BURRITT & PROVENZA, 1990), horses (PFISTER et al., 2002), and goats (GORNIK et al., 2008; OLIVEIRA et al., 2014) to avoid the ingestion of toxic plants. The aversion persisted for at least 3 months in the treated calves, and it is possible that it may have not extinguished for a long period of time. RALPHS reported that cattle averted to toxic but palatable *Delphinium* species retained the aversion for at least 3 years (RALPHS, 1997).

We also reported that calves eat *S. bonariense* leaves after 10 days when kept under a restrictive diet (50% of ME), thus eventually overcoming the neophobia against this shrub, in a similar manner to what likely occurs during spontaneous poisoning during the dry season or during periods of forage shortages due to drought or overgrazing (VERDES et al., 2006). This suggests that on farms where cattle are periodically poisoned with this plant, cattle must have a reduced allotment of desirable forage for about 10 days before they will voluntarily consume *S. bonariense* while grazing. Therefore, cattle producers with this troublesome weed need to define a specific strategy to prevent its consumption by cattle during the dry season when forage is often scarce, and avoid overgrazing during other seasons to prevent potential intoxication. The implementation of a conditioning aversion protocol with LiCl could be a useful tool to reduce or eliminate

*S. bonariense* poisoning in this and other regions in South America where the plant causes intoxication.

In our experimental conditions LiCl produced an aversion to *S. bonariense* in calves. Two doses were sufficient for the calves to associate the adverse effects from LiCl with this specific poisonous shrub. Other studies suggested that in some cases, it is necessary to repeatedly dose with LiCl to produce an aversion (BURRITT & PROVENZA, 1990). In this study, only a second reinforcing dose was needed for calves to develop a strong aversion against *S. bonariense*. Conditioned aversion appears to be a potentially useful tool to reduce or eliminate consumption of *S. bonariense* in calves. It could be a valuable strategy for farms affected by spontaneous intoxication in cattle from *S. bonariense*. Future research is necessary to determine the length of time that calves will maintain the aversion against *S. bonariense*. Further study will also be required to determine if non-naïve cattle that have ingested the plant while grazing, but are not yet intoxicated, can be averted (PFISTER et al., 2007) to *S. bonariense*. Future aversion studies should include goats and sheep as animal models because they are also intoxicated by this plant (VERDES et al., 2012).

## CONCLUSION

A reduction in the daily energy intake to 50% of requirements for 10 days induced calves to consume the unpalatable *S. bonariense*. Two doses of LiCl produced an aversion to *S. bonariense* in calves, which persisted for at least 3 months.

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## BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The experiment was approved by the Institutional Animal Care and Use Committee of Facultad de Veterinaria (CEUA-FVET, UdelaR), No. CEUAFVET-TG 02 – Exp. 111130-000273-14.

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